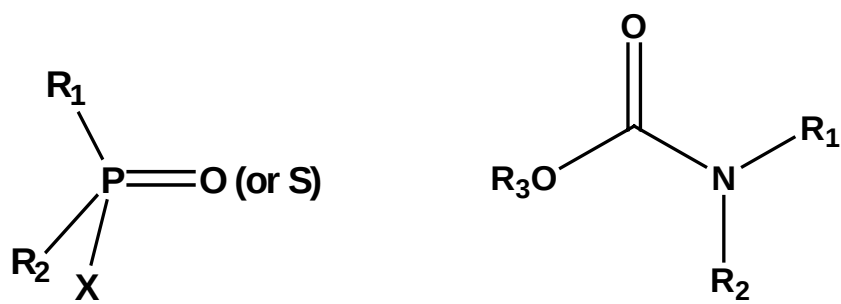


SCIENCE GROUP

A CRITIQUE OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY'S (US EPA) GROUPING OF INSECTICIDAL ORGANOPHOSPHATES AND N-METHYL CARBAMATES INTO COMMON MECHANISM GROUPS



Organophosphates & N-Methyl Carbamates

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SCIENCE GROUP

A CRITIQUE OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY'S (US EPA) GROUPING OF INSECTICIDAL ORGANOPHOSPHATES AND N-METHYL CARBAMATES INTO COMMON MECHANISM GROUPS

INTRODUCTION

1. At the meeting of the Science Group held in March 2003, issues regarding the establishment of common mechanism groups (CMGs) for UK registered pesticide and veterinary medicine substances were discussed. A method for grouping these substances into common mechanism groups was considered and accepted by the Science Group. The United States Environmental Protection Agency (US EPA) (together with the International Life Sciences Institute [ILSI]) devised this process and it is outlined in Science group paper (**SciGroup/2003/02**). The Science Group were informed that the US EPA had already begun the process of grouping substances into CMGs and had established a CMG for the organophosphates (OPs) and carbamates. Due to the resource implications it was agreed that the most sensible approach would be to evaluate the way in which these two common mechanism groups were established with a view to adopting the groups should the decisions made be acceptable to the Science Group.
2. The paper presented here outlines the critical decisions made by the US EPA in establishing the OP and carbamate CMG. It also presents background information on the major areas that form part of the assessment of CMG: structural property; mechanism of toxicity; toxic effects; and pesticidal action.
3. In the United States the Food Quality Protection Act (FQPA) of 1996 requires the US EPA to consider potential human health risks to multiple pesticides that act by a common mechanism of toxicity, from all dietary and non-dietary routes of exposure. To assess such risks a cumulative risk assessment is required and the first stage of this process is to identify CMGs of pesticides. Accordingly the US EPA has identified a number of CMGs, of which the OP and carbamate pesticides were amongst the first to be considered. This was because OP pesticides and carbamates had been assigned priority for cumulative toxicity testing.
4. In 1998 the US EPA established the OPs as a CMG on the basis of their shared ability to inhibit acetylcholinesterase (AChE) by phosphorylation of the serine hydroxyl residue located in the active site of the enzyme. This is the critical step that leads to a common spectrum of toxic effects associated with cholinesterase inhibition, the most sensitive toxic effect of the OPs. In 2001 the US EPA established the N-methyl carbamate pesticides as a separate CMG on the basis of their similar structural characteristics, shared ability to inhibit the AChE enzyme by carbamylation of the same serine hydroxyl residue in its active site and consequential adverse effects on cholinergic function (cholinergic toxicity).
5. The US EPA published a guidance document (in 1998) outlining a mechanism by which CMGs were to be identified which they have followed step by step during the establishment of some CMGs (e.g. the triazines). However in the case of the N-methyl carbamates and the Ops, the wealth of information already available on these pesticides meant that they did not require such a rigorous assessment for

establishment as a CMG. Nevertheless the grouping of these two classes of pesticides was to a certain extent concordant with this guideline.

US EPA/ILSI CONSIDERATION OF THE OP AND N-METHYL CARBAMATE INSECTICIDES

6. Although the guidance document outlining an approach for grouping substances specifies a number of factors that need to be considered when identifying a CMG, the US EPA felt that in the case of the OP insecticides the only scientific question that needed to be answered was whether AChE inhibition alone was sufficient evidence of a common mechanism of action or whether factors such as absorption, distribution, metabolism and excretion (ADME) should also be considered.
7. As a result of their varying chemical structures, different OPs will have: a) varying potencies; and b) varying toxicokinetics (ADME), so the US EPA considered these parameters during internal meetings, meetings with advisory panels and through public consultations.
8. The first critical decision made was that cholinesterase inhibition alone did constitute a mechanism of action for cholinergic toxicity. This meant that OP insecticides capable of inhibiting cholinesterase enzymes should be grouped into a CMG for this specific effect. The scientific reasoning behind this decision was that cholinesterase inhibition is the critical step that leads to an array of signs and symptoms characteristic of cholinergic toxicity, the severity of which is dependant on dose and potency. The OP compounds would not be able to elicit these effects if they, or their metabolites, lacked cholinesterase inhibiting activity. The spectrum of effects elicited by each OP is a reflection of differing toxicokinetics and is viewed as an indicator of the degree of toxicity not the mechanism of toxicity. The ADME of each specific compound will play an important role at a later stage of the cumulative risk assessment for the OP CMG. There are some OP compounds that do not inhibit acetylcholinesterase or produce any signs of cholinergic toxicity. For example, the herbicide glyphosate, the plant growth regulator ethephon, and the fungicide fosetyl-aluminium were not grouped in the anticholinesterase CMG
9. During the US EPA evaluation of the carbamate pesticides, the critical factors considered were the structural requirements for cholinesterase inhibition, and the spectrum of toxic effects elicited by each member of the carbamate pesticides. The latter was particularly important because the most sensitive toxicological effect of some of these pesticides involved cholinesterase inhibition whilst others did not. Evaluation of the chemical structures of the carbamates led to the establishment of three groups: 1) methyl carbamates; 2) thiocarbamates; and 3) dithiocarbamates. A review of the main toxic effects and mechanism of toxicity of these groups led the US EPA to establish the N-methyl carbamates as a common mechanism group on the basis of anticholinesterase activity.
10. The decision to group the N-methyl carbamates and the OPs into separate common mechanism groups has been the subject of debate, particularly because it goes against the recommendations of expert advisory panels consulted by the US EPA. Furthermore it is questionable if the decision to group these classes of substances is concordant with the CMG grouping guidelines.

ISSUES FOR THE SCIENCE GROUP TO CONSIDER

11. The mechanism of toxicity of the N-methyl carbamates and the OPs have been outlined in annexes 1 and 2 and the decisions made by the US EPA in grouping these two classes of substances are set out above. The differences between the kinetics of carbamylation and phosphorylation have formed the basis for the US EPA's decision to group the two classes of pesticides independently. The Science Group will need to decide if these differences provide a scientifically valid justification for grouping the N-methyl carbamates and the OPs separately.

12. Upon review of the information outlined in this paper the Science Group is asked to consider the following:

- *Is cholinesterase inhibition alone the only important mechanism of action for the anticholinesterase OPs and N-Methyl carbamates?*
- *Can carbamylation and phosphorylation of cholinesterase enzymes be considered a common mechanism of toxicity with regard to the spectrum of toxicological effects elicited, and can any differences in the pharmacokinetics of the reactions be accounted for at a later stage of the cumulative risk assessment?*

Or:

- *Are differences in the pharmacokinetics of carbamylation and phosphorylation reactions sufficient basis for independently grouping compounds that inhibit cholinesterase?*
- *An OP insecticide, phoxim, authorised for use in the UK but not the US was not evaluated during the establishment of the US OP CMG. The Science Group will need to consider grouping this compound with the other 'grouped' OPs.*

13. **Guidance for Question iii:** To make this choice, the Science Group needs to be satisfied that the pharmacokinetics of cholinesterase inhibition is a critical step in the mechanism of toxicity of these compounds. Thus, any differences in the pharmacokinetics also equate to a difference in the mechanism of toxicity. If this is decided, then the two classes of compound will not meet the criteria required for the establishment of a CMG and as a consequence should be grouped separately.

ANNEX 1: ORGANOPHOSPHATE COMMON MECHANISM GROUP

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STRUCTURAL FEATURES OF THE ORGANOPHOSPHORUS ESTERS REQUIRED FOR ANTICHOLINESTERASE ACTIVITY.

14. The OP pesticides are esters of phosphoric acid ($P=O$) or phosphorothionic ($P=S$) acid.



Figure 1. Phosphoric and Phosphorothionic acids.

15. In terms of their structure, the OP pesticides are one of the most diverse classes of pesticidal substances. They do, however, conform to a general structure that is essential for anticholinesterase activity (Fig. 2), consisting of a phosphorus atom surrounded by two alkyl/alkoxy groups and a third group, the leaving (X) group.

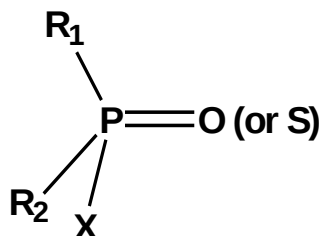


Figure 2. General Structure of the Organophosphorus Insecticide

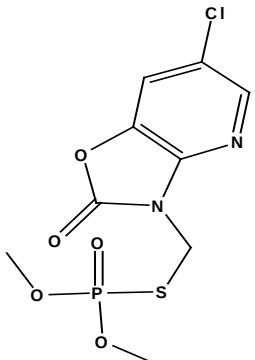
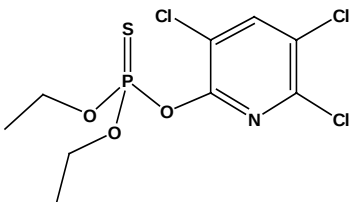
16. In most cases, both R groups in pesticidal OPs are the same, either both methoxy or both ethoxy, although one or two have other R groups. The X substituent is known as the leaving group because it is displaced when the OP binds to the AChE serine hydroxyl group. The X group can be any one of a much broader range of chemical structures than the R_1 and R_2 groups and is designed by pesticide manufacturers to confer reactivity to the P-X bond of the pesticide. Specific chemical structures of X are also selected for the purposes of modifying the physical/chemical properties of the molecule in order to modify its pharmacokinetics and pharmacodynamics (e.g. biological activity at AChE).
17. The structural diversity of the OP compounds and the variations in the spectrum of cholinesterase activity between the compounds are largely due to the X group. The electronegative and steric properties of X are primarily responsible for the reactivity of the OP and determine the degree of its acute toxicity. As will be discussed later, the phosphorus atom which is intrinsically stable has to be converted into a reactive state (positively charged) before the AChE serine hydroxyl residue can

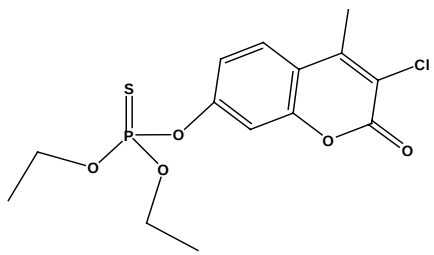
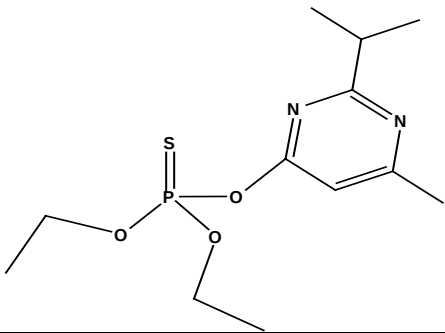
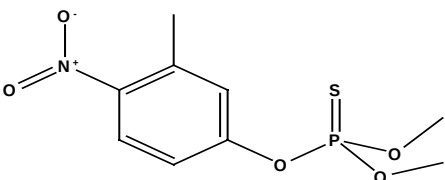
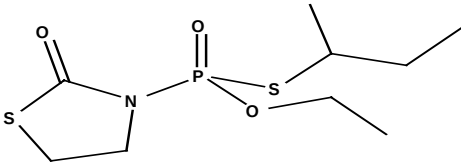
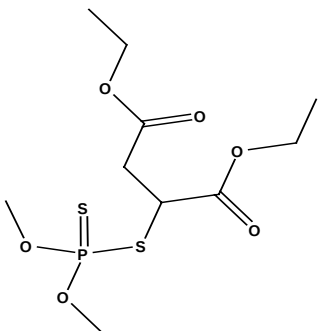
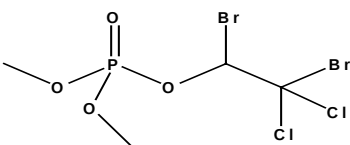
attack it via nucleophilic forces of attraction. The X group converts the phosphorus atom into a reactive state (i.e. reactive enough to phosphorylate the enzyme) by withdrawing its electron(s). The steric properties of X will also modify the acute toxicity of the compound. Moieties that are large and bulky (e.g. the S-1,2-bis(ethoxycarbonyl)ethyl group in malathion) makes for poorer cholinesterase inhibition than smaller chemical groupings (e.g. fluorine in nerve agents).

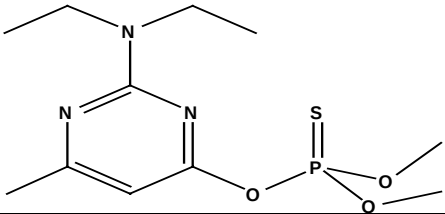
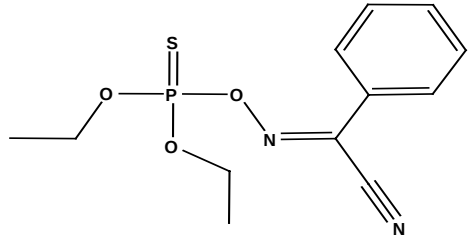
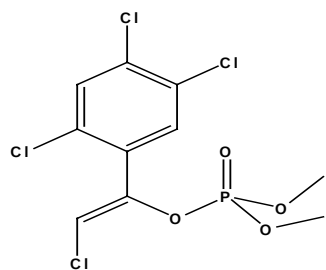
18. Generally, the smaller and more electronegative the group, the higher the degree of acute toxicity. The P=O/P=S bond also plays an important role in enhancing the electropositive potential of the phosphorus atom. The phosphorothionate esters are poor cholinesterase inhibitors compared to the phosphate esters because the sulphur atom double bonded to the phosphorous atom is far less electronegative than the oxygen atom. This makes the P=S bond less polarized than the P=O bond thus resulting in a phosphorus atom with low reactivity and minimal, if existent, capacity to inhibit the enzyme. The fact that the phosphorothionates are used as insecticidal compounds because they are converted by mixed function oxidases present in insects into phosphates through the metabolic oxidation of P=S to P=O (Fukato, 1990). Oxidative desulfuration also occurs in mammals, so that the phosphorothionates can cause anticholinesterase toxicity in mammals.

19. Table 1 shows the chemical structures of UK registered OP insecticides.

Table 1. Chemical Structures of UK Registered OP insecticides

Active	Uses	Molecular Structure
Azamethiphos (P, V, B)	Nematicide/Insecticide	
Chlorpyrifos (P, B)	Insecticide	
Chlorpyrifos methyl (P)	Insecticide	

Coumaphos (V)	Insecticide	
Diazinon (V)	Insecticide	
Fenitrothion (B)	Insecticide	
Fosthiazate (P)	Insecticide	
Malathion (P)	Insecticide	
Naled (V = Companion species use only)	Insecticide	

Pirimiphos-methyl (P, B)	Insecticide, Wood preservative	
Phoxim (V)	Insecticide	
Tetrachlorvinphos (V = Companion species use only)	Insecticide	

P = Pesticidal Use

V = Veterinary Use

B = Biocidal Use

MECHANISM OF TOXICITY OF THE ORGANOPHOSPHATES

20. To understand the toxicological effects of the anticholinesterases it is important to first understand the physiological role of acetylcholine (ACh).

Physiological Role of the Neurotransmitter ACh.

21. ACh binds to and activates two types of cholinergic receptors, muscarinic and nicotinic, which are structurally unrelated (Figure 3).

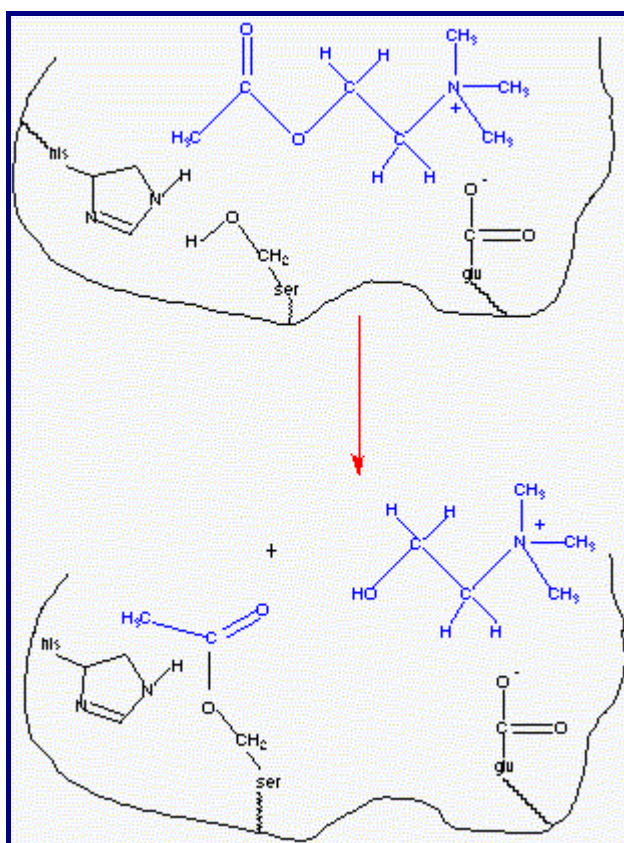


Figure 3. Schematic diagram of an acetylcholine molecule being hydrolysed in the active site of the acetylcholinesterase enzyme.

22. They are both widely distributed throughout the peripheral nervous system and central nervous system and mediate numerous functions as a result of their presence on a wide variety of organs. Muscarinic receptors are coupled via G-proteins to the enzymes adenylate cyclase or phospholipase C. Activation of M₁ receptors, the most important type of muscarinic receptor, leads to an increase in Ca⁺⁺ conductance of local ligand-gated Ca⁺⁺ channels. Nicotinic receptors are part of a superfamily of ligand-gated ion channels, with γ-aminobutyric acid A (GABA_A), glycine and other receptors.

23. The activity of ACh at these sites has to be tightly regulated in order to maintain the normal functioning of these organs. An important means of controlling this activity is the rapid degradation of the neurotransmitter by AChE. Failure to achieve this results in accumulation of ACh at neuroeffector sites. This disrupts the function of these organs and leads to the adverse effects summarised in Table 2. The spectrum of effects elicited by a specific anticholinesterase compound will depend

on the distribution of the OP within the body and the type of receptors affected by the accumulation of the neurotransmitter.

Table 2. Effects of acetylcholine accumulation and types of receptors involved.

	Organ/cell	Receptor type	Effects
Central nervous system	Cholinergic tracts	Muscarinic, nicotinic	Confusion and apprehension, convulsions, coma
Peripheral nervous system	Neuromuscular junction	Nicotinic	Muscle fasciculation and weakness
Autonomic nervous system	Parasympathetic effector organs	Muscarinic	Overactivity of the salivary, bronchial and sweat glands, contraction of smooth muscle in the eye, respiratory tract, gut and the cardiovascular system, bradycardia
	Sympathetic ganglia	Nicotinic	Tachycardia, hypertension

24. *Central Nervous System (CNS)*: Cholinergic neurotransmission within the CNS plays a central role in memory, cognitive performance, vigilance, locomotor activity, regulation of body temperature, respiration cardiovascular function, EEG activity, cortical blood flow, and pain perception (ACNP, 2000).

25. *Peripheral Nervous System (PNS) & Autonomic system*: ACh is also a neurotransmitter in the periphery, including at the neuromuscular junction and in the autonomic nervous system. In the latter, ACh is involved in neurotransmission at the ganglia of the sympathetic nervous system and at effector organs in the parasympathetic nervous system. At the neuromuscular junction and the sympathetic ganglia the receptors are nicotinic, whereas at the parasympathetic effector organs the receptors are muscarinic.

Physiological Role of AChE

26. AChE is a ubiquitous enzyme expressed in a wide range of mammals and invertebrates (e.g. insects). It is localised within the vicinity of the nicotinic receptors, e.g. tethered to the basement membrane overlying the postsynaptic membrane or motor endplate where the nicotinic receptors are situated. This close approximation to the nicotinic receptors means that the enzymes can rapidly hydrolyse each ACh molecule before they can make interactions with the receptors. AChE is also found in association with muscarinic receptors, where it performs a similar role in hydrolysing ACh. AChE breaks down acetylcholine to choline and acetic acid (Figure 3) and is itself regenerated, freeing it (within an extremely rapid time frame) to hydrolyse other ACh molecules. The speed at which the acetylation and regeneration of the enzyme takes place is absolutely essential for the regulation of cholinergic neurotransmission. A reduced capacity to degrade and remove ACh from the synaptic cleft causes the accumulation of ACh, prolonged activation of nicotinic and muscarinic receptors and consequent cholinergic toxicity.

Biochemistry of OP Anticholinesterase Activity

27. Anticholinesterase activity induced by an OP ester is a two-step process, the first being the formation of an enzyme inhibitor complex (Step 1 in Figure 4) and the second being the phosphorylation of the enzyme (Step 2 in Figure 4).

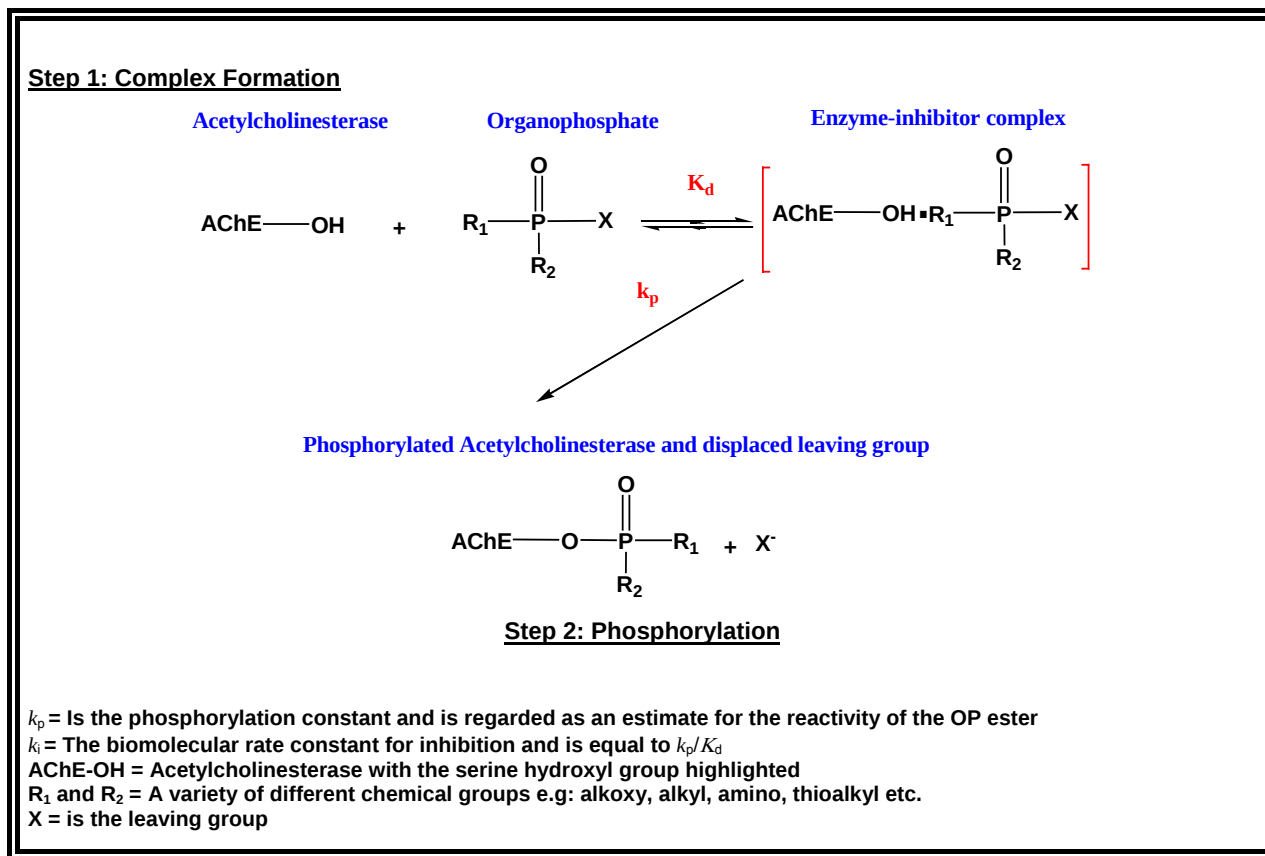


Figure 4. Scheme for equation of OP inhibition of AChE (Fukuto, 1990)

28. The phosphorylated enzyme produced by OP inhibition is much more stable than the acetylated enzyme produced when AChE hydrolyzes its natural substrate, Ach. However, with one or two exceptions measurable but slow hydrolysis of phosphorylated AChE occurs. As the vast majority of pesticides contain two dimethoxy or two diethoxy groups, the usual inhibited enzyme is either a dimethoxyphosphoryl derivative or a diethoxyphosphoryl derivative. Dimethoxyphosphorylated enzyme hydrolyses faster than diethoxyphosphorylated enzyme. Clinically insignificant hydrolysis occurs with certain OPs such as diisopropylphosphorofluoridate and also where ageing has occurred. Ageing is a process which renders the inhibited enzyme refractory to reactivation and consists of loss of an alkyl group to leave a monoalkylphosphoryl enzyme. Ageing is a serious problem with the nerve agent soman, but is a potential problem in patients with pesticide poisoning where treatment has been delayed. Rates of synthesis of enzyme *de novo* can be quite rapid and this may contribute to reappearance of enzyme activity after inhibition by OPs, especially where reactivation by hydrolysis is slow or clinically insignificant (see reviews by Wilson, 1992 and Marrs, 2001).

29. While the AChE remains phosphorylated, it is unable to perform its natural function of hydrolysing ACh. This results in overactivity at sites of cholinergic neurotransmission.

30. Electrophilicity of the phosphorus (P) atom is central to the biological activity of OPs. To phosphorylate the nucleophilic serine hydroxyl residue located in the active site of the cholinesterase enzyme the P atom has to be highly electrophilic. The P atom, however, is not intrinsically reactive and is dependant on the presence of: 1) a double bond with an oxygen atom; and 2) attachment of electronegative groups to be converted to a highly electrophilic state which enhances the reactivity of the compound. The phosphorus atom is converted to a highly positively charged state by the substituent group (attached at X) withdrawing one of the electrons. Once the atom is in this highly charged state, the AChE serine hydroxyl moiety that is itself negatively charged (by virtue of the oxygen atom), will attack the charged phosphorus atom and form a stable bond. If the substituent group of an OP is very weakly electronegative or is electropositive, it will not be able to withdraw the electron from the phosphoryl atom and the atom will not be sufficiently reactive to form the bond required to inhibit AChE. Therefore, the more electronegative the substituent group the more potent the inhibition.

TOXIC EFFECTS OF THE OP INSECTICIDES

Acute effects

31. The effects of OP anticholinesterases can be divided into peripheral effects (mediated by muscarinic receptors), peripheral effects (mediated by nicotinic receptors) and central effects (mediated by both type of receptor).

Mild to moderate toxicity

32. Muscarinic receptors are found in parasympathetic effector organs. Accumulation of ACh at such sites causes overactivity of the salivary, bronchial and sweat glands, bronchorrhea and sweating. Contraction of the smooth muscle in the eye (iris), respiratory tract, gut and the cardiovascular system produces myosis (constriction of the pupil), tightness of the chest, abdominal cramps and bradycardia and hypotension. Nicotinic receptors are found in the ganglia of the sympathetic nervous system and at the neuromuscular junction. Accumulation of ACh at sympathetic ganglia may produce effects that are the opposite of parasympathetic effects. This is especially the case in the cardiovascular system where brady- or tachycardia and hypo- or hypertension may be seen. Nicotinic effects on neuromuscular function may include muscle fasciculation and weakness. Accumulation of ACh in the CNS may produce confusion and apprehension.

Severe toxicity

33. In addition to the effects outlined above, severe toxicity is characterised by symptoms associated with CNS toxicity including restlessness, emotional lability, ataxia, lethargy, mental confusion, loss of memory, convulsions, generalised weakness, cyanosis and coma. Respiratory paralysis, which may be of central or peripheral origin, is the main cause of death in fatal cases of poisoning (Marrs and Dewhurst, 1998). Fatal cardiac arrhythmias may occur including *torsade des pointes*.

34. Most of these effects are reversible. Mild to moderate toxicity is usually reversed without permanent damage after a few days to weeks when the phosphorylated enzyme is spontaneously reactivated. Reactivation is usually produced by hydrolysis of the enzyme/OP complex. In more severe cases of toxicity recovery is dependant upon rapid treatment with atropine and a pyridinium oxime such as pralidoxime. In some cases of severe non-fatal toxicity permanent changes in electrophysiological and clinical parameters may occur in the CNS.

Delayed and Chronic Effects

35. One of the main differences between OPs and the AChE inhibiting carbamates are the chronic effects outlined below which are only induced by the OPs.

Intermediate Syndrome (IS):

36. Intermediate Syndrome (IS) is a paralytic syndrome characterised by neurological signs that manifest between 24-96 hours (1-4 days) after an acute cholinergic crisis. The main toxicological effect is muscle weakness, primarily affecting muscles that are innervated by cranial and arm nerves. These muscles are the neck flexors, proximal limb muscles, facial muscles and the respiratory muscles. Thus IS is a proximal muscle paralysis. Mechanisms proposed for this phenomenon include: post-synaptic block of neuromuscular transmission; persistent inhibition of blood AChE activities; insufficient oxime therapy; and, less likely, necrotising myopathy. Compounds known to cause this effect include: diazinon; dichlorvos; dimethoate; fenthion; methamidophos; monocrotophos; omethoate; parathion; and phoxim .

Organophosphate-Induced Delayed Polyneuropathy (OPIDP):

37. This is a mixed central and peripheral neuropathy and there are both sensory and motor components. It is characterised by an initial flaccidity (muscle weakness in the arms and legs giving rise to a clumsy, shuffling gait), which progresses to spasticity, hypotonicity, hyperreflexia, clonus (an effect characterised by rapid contraction and relaxation of a muscle), and abnormal reflexes. These symptoms are indicative of damage to pyramidal tracts and a permanent upper motor neurone syndrome. In some patients, recovery has been reported to be limited to the arms and hands. The sensory component consists of a of "stocking" type numbness. Inhibition of the neuronal non-specific carboxylesterase, neuropathic target esterase (NTE) is associated with OPIDP.
38. The physiological role of this enzyme has not yet been fully elucidated, however it is thought to be involved in lipid metabolism in neurones. Inhibition of NTE is a reliable indicator of OPIDP poisoning, the clinical effects of which appear approximately 20 days after the initial exposure. Acute exposures that result in >70% inhibition of NTE will normally be followed by OPIDP, with ataxia being observed some 7 to 14 days following treatment. Compounds known to cause this effect include: chlorpyrifos; fenthion; leptophos; methamidophos; mipafox; omethoate; parathion; trichlorfon; trichloronate; and tri-O-tolyl phosphate (TOTP).

Chronic Organophosphate-Induced Neurological Defect (COPIND):

39. While there is little doubt that acute severe poisoning, especially if convulsion occur, can produce long term nervous system deficits, whether lower doses can or not continues to be a matter of controversy

Toxicological Endpoints Assessed by the US EPA to Determine Cholinesterase Inhibition During the Grouping Of the Organophosphates

40. The three key endpoints considered by the US EPA and its Office of Pesticides Program (OPP) as determinants of carbamates/OPs classification as cholinesterase inhibitors were:

- Effects on cholinergic function (physiological and behavioural/functional effects).
- Neuronal (CNS and PNS) acetylcholinesterase inhibition.
- Blood (RBC and Serum) cholinesterase inhibition.

41. Adverse effects on cholinergic function can be determined by observation of symptoms (a condition defined as a departure from normal function reported by the person experiencing and reporting the condition and clinical signs in:

- PNS:
 - Smooth muscle contractions such as abdominal cramps
 - Skeletal muscle twitching which at acutely toxic doses can lead to flaccid paralysis
 - Glandular secretions (e.g. increased sweating, lacrimation and salivation)
 - Muscle twitching and weakness.
- CNS:
 - Effects on learning, memory and other behavioural parameters
 - At high doses, respiratory depression and coma.

PESTICIDAL ACTION OF THE ORGANOPHOSPHATES

42. For many pesticides the biomolecular target (e.g. structural proteins, receptors, ion channels, enzyme systems) in the target species is also present in humans. In the case of Ops, dysregulation of the cholinergic system through the alteration of synaptic levels of ACh is the basic mechanism of pesticidal action of the OPs. Sufficient dysregulation perturbs the normal functioning of tissues dependant on cholinergic neurotransmission and is lethal to insects.
43. Significant differences between a human's capacity and the target pests capacity to detoxify such compounds ensures the safe use of the pesticide. Also, in insects the nervous system is more accessible to toxicants than the mammalian nervous system, which is protected by the blood brain barrier (BBB). In addition mammals have detoxification systems in place which protect against low level exposure to OPs thus ensuring that doses of anticholinesterases which are lethal to the insect does not present the same risk to humans. Mammals have large and significant amounts of carboxylesterases in their serum, which will rapidly degrade unbound

molecules of OPs present in the systemic circulation. This prevents the residues of the compounds from accumulating within the systemic circulation of humans and reaching a lethal level of exposure. Insects only synthesise a small amount of carboxylesterases, so low that levels of OPs overwhelm their cholinergic system. Thus without an adequate detoxification system in place to reverse the effects of the OPs, concentrations that do not have a significant effect on humans are lethal to insects. The safe use of OP compounds is thus heavily reliant on the existence of these detoxification systems.

US EPA OFFICE OF PESTICIDE PROGRAMS & ILSI RISK SCIENCES INSTITUTE DEVELOPMENT OF THE OP CMG

44. The definition of a mechanism of toxicity drafted by the US EPA in 1997 is as follows:

“A mechanism of toxicity is described as the major steps leading to an adverse health effect following interaction of a pesticide with biological targets. An understanding of all steps leading to an effect is not necessary, but identification of the crucial events following chemical interaction is required to describe a mechanism of toxicity”.

45. The criteria agreed by the US EPA for establishing a CMG was:

“ two or more chemicals may act via a common mechanism of action if they:

- cause the same critical effect;**
- act on the same molecular target at the same target tissue;**
- act by the same biochemical mechanism of action, or share a common toxic intermediate”.**

46. The ILSI Risk Sciences Institute (RSI) convened a Working Group of experts to determine whether OP insecticides act by a common mechanism of toxicity if they inhibit AChE.

47. To a large extent the OP pesticides do conform to these criteria, with them or their metabolites sharing a broadly similar chemical structure, which confers moderate to high anticholinesterase activity. They bind to and inhibit the cholinesterase enzymes through the same biochemical reaction e.g. phosphorylation of the serine hydroxyl residue located in the cholinesterase active site and cause accumulation of ACh at neuro-effector, neuro-neuro, and neuro-muscular junctions. There are, however, differences in the spectrum of cholinergic effects of individual cholinesterase inhibiting OPs which suggests differences in the within this class of pesticide.

48. In order to determine whether the OP pesticides do act by a common mechanism of toxicity, the Working Group felt it necessary to investigate the reasons why individual substances induced different spectra of cholinergic toxicity. This would highlight the existence of slight mechanistic differences that might be shared by certain anticholinesterase OPs. The Working Group generated a testable hypothesis that anticholinesterase OP compounds act by a common mechanism of toxicity.

DEVELOPMENT OF HYPOTHESIS

49. The Working Group tested the hypothesis that cholinesterase inhibiting OP pesticides act by a common mechanism of action by first considering whether there were any aspects of OP toxicity that indicate some OPs could interact with target sites by a mechanism different to other OPs. To decide this they considered the potential for OP pesticides to:

- Interact specifically with muscarinic or nicotinic receptors
- Differ with respect to metabolic activation and detoxification
- Differ regarding to distribution to CNS, peripheral, sympathetic and parasympathetic tissues.

50. This was tested by considering:

“OP Pesticides can be separated into subgroups based on whether or not:

- a) **They require metabolic activation to confer anticholinesterase activity.**
- b) **They have toxicological actions which operate instead of, or in addition to, cholinesterase inhibition.**
- c) **They are activated or deactivated by different enzymes located in different parts of the body.**
- d) **There is differential action on muscarinic versus nicotinic receptors**
- e) **There is differential distribution in the body, and consequent action on different target tissues.**
- f) **They act solely on the peripheral nervous system, versus action solely on the CNS.”**

51. After brief consideration, some of these hypotheses were rejected outright whilst the rest were considered in more detail.

OP Pesticides can be separated into subgroups based on whether or not they require metabolic activation to confer anticholinesterase activity.

52. This hypothesis was rejected. The two assumptions that formed the basis of this hypothesis was:

- ***The process of metabolic activation would delay the onset of inhibition of AChE compared to the immediate effects of a direct acting compound.***

- The Working Group agreed that any delay in the onset of AChE inhibition would only be important if there were simultaneous exposures to multiple compounds at a distinct point in time. In reality humans are probably exposed to pesticide residues via numerous routes of exposure for overlapping periods of days to weeks.
- ***A compound that requires metabolic activation would be subject to biotransformation-mediated interactions that could alter the dose-response relationship of the compound***
- The Working Group felt that the metabolic processes that exist in mammals were not expected to alter the mechanism of toxicity of the parent OP or its metabolites.

53. It should be noted that apart from a few specific compounds, most OP insecticides require metabolic activation to their oxon derivatives to acquire cholinesterase inhibiting activity. Direct-acting compounds are already oxons and will act in the same way (e.g. phosphorylate cholinesterase) as the metabolically activated compounds when delivered to their target. OPs that require metabolic activation to 'switch on' anticholinesterase activity are activated by the same set of distinct enzymes systems (mixed function oxidase enzymes). None of them appear to have an activation pathway that could alter the mechanism of toxicity of these compounds.

OP Pesticides can be separated into subgroups based on whether or not they have toxicological actions operating instead of, or in addition to, cholinesterase inhibition.

54. The Working Group tested this hypothesis by looking for indicators that inhibition of AChE does not correlate with toxicity in the way that might be expected. For example, a highly potent AChE inhibitor would be expected to exert a greater degree of toxicity than a weak inhibitor at an equivalent dose. Deviations from this scenario might be expected to be a consequence of an additional mechanism of toxicity aside from cholinesterase inhibition, and could lead to a sub-grouping of the OP insecticides. A variety of pharmacokinetic data could be used to assess toxic potency:

- IC_{50} = concentration of the OP compound that inhibits 50% of AChE activity *in vitro* under specified conditions.
- k_i = the bimolecular inhibition rate constant
- = the spontaneous reactivation rate of the phosphorylated AChE
- ED_{50} = the dose that will cause a given functional change in 50% of intact animals
- LD_{50} = the median lethal dose in intact animals
- K_a = First-order rate constant for ageing

55. However a search through the available database for evidence to support this hypothesis proved unsuccessful because of inconsistencies between species studied. For this reason the hypothesis was rejected.

OP Pesticides can be separated into subgroups based on whether they are activated or deactivated by different enzymes located in different parts of the body.

56. OP insecticides are activated and deactivated by the P450 cytochrome oxidase family of enzymes (Table 3) which has a number of members (30 or more families and subfamilies) that differ in structure and specificity of the reactions catalysed. Different isoforms of the P450 enzymes are differentially distributed throughout different organs and tissues in the body. Therefore, rates of activation and deactivation of the OP insecticides is likely to differ from tissue to tissue.

57. To divide the OPs into groups on the basis of differential metabolism, the specific isoforms involved in the metabolism of the OPs and their tissue distribution would need to be known. However much of this type of information is currently unavailable. For this reason the Working Group felt that there was not enough information to justify separating the OP compounds into sub-groups. In addition, it is well established that a number of other enzyme families (Table 3) are involved in the metabolism of OP compounds, yet there is insufficient information available about the tissue distribution of these enzymes. Sub-grouping the OPs according to differences in site-specific metabolism cannot be considered until this type of data becomes available.

Table 3. Enzymes involved in the metabolism of the Ops

ENZYMES THAT METABOLICALLY ACTIVATE OP INSECTICIDES	
Microsomal oxygenases – oxidative disulphuration	P450 and the FMO enzyme systems catalyse reactions that increase the anticholinesterase activity of the OP. In humans the CYP 3A4 and CYP 3A5 enzymes converts parathion into its toxic metabolite paraoxon, a reaction that increases the AChE inhibiting activity of the insecticide by a thousand fold.
ENZYMES THAT METABOLICALLY DEACTIVATE OP INSECTICIDES	
A-esterases (Paraoxonase, PON)	PON is an enzyme whose natural role seems to be in lipid metabolism. It can also hydrolyze a variety of OPs
Carboxylesterases (including B-esterases)	Carboxylesterases are present in mammalian plasma and tissues such as the liver, lungs, kidney, brain intestines, gonads and muscle. They play an important role in reducing the toxicity of thion OPs by binding to the OPs and hydrolysing them, which in turn reduces the amounts of free OP molecules available to inhibit the cholinesterases. Depending on the specific OPs the carboxylesterases either hydrolyse the OP (and is subsequently regenerated) or becomes inhibited by the OP.
Microsomal Oxygenases – 1.) Oxidative O- and N- dealkylation, and dearylation. 2) Thioether oxidation. 3) Side chain oxidation	P450 and the FMO enzyme systems also catalyse reactions that decrease the anticholinesterase activity of the OP.

OP Pesticides can be separated into subgroups based on whether there is differential action on muscarinic versus nicotinic receptors

58. This hypothesis is based on the idea that some OP compounds will preferentially stimulate signs of cholinergic toxicity related to the activation of either the nicotinic or muscarinic receptors or some other unknown effect. It is known that some OP compounds can bind directly to these receptors and through allosteric changes in the receptor channel conformation alter the degree of receptor activation. If specific compounds can trigger muscarinic only or nicotinic only signs of cholinergic toxicity then there would be sufficient justification to sub-group the OP compounds on this basis.
59. The Working Group looked at available data to find if there was a direct relationship between a specific OP and toxic effects associated with these receptors. A study conducted by Sheets *et al* (1997), in which a functional observation battery test (Table 4) was conducted on rats treated with one of six OP insecticides, provided strong evidence that a receptor-specific type of cholinergic toxicity does not exist among OP compounds. In this study the OPs tested were sulprofos, tebupirimphos, disulfoton, azinphos-methyl and trichlorfon. They caused muscle fasciculations, tremors, and perineal staining. Methamidaphos impaired the righting reflex of the rats tested. The results show that the pesticides cause both muscarinic and nicotinic receptor overstimulation. The Working Group assessment of data from standard laboratory animal studies conducted with the other OP compounds supported the observations from the Sheets *et al* (1997) study.

Table 4. Signs and Symptoms Assessed in a Functional Observation Battery Screen

	Clinical signs and symptoms assessed for receptor activation	
	Muscarinic	Nicotinic
Motor Activity		Observation of muscle fasciculations and tremors. Decrements in aerial righting (righting reflex). Measured in an automated activity recording device. Grip performance
Autonomic Nervous System	Lacrimation. Salivation. Frequency of urination Presence or absence of diarrhoea. Constriction of the pupil of the eye in response to light or a measure of pupil size.	

OP Pesticides can be separated into subgroups based on whether there is differential distribution in the body, and consequent action on different target tissues.

60. The Working Group felt that there was insufficient data within the available pesticide/ veterinary authorisation dossiers to support or reject the hypothesis of a specific regional distribution of the OPs and consequent tissue specific effects that may arise from this kind of exposure. The hypothesis was rejected due to the lack of supporting data.

OP Pesticides can be separated into subgroups based on whether there is action solely on the peripheral nervous system, versus action solely on the CNS.

61. This hypothesis was based on the potential for specific OPs to be selectively distributed to and transported across the BBB to exert effects within the CNS, or alternatively be unable to cross the BBB in which case the toxicity of the compound would be restricted to the peripheral nervous system. OPs are highly lipophilic and most are fully distributed to all parts of the body including the CNS.

62. The Working Group reviewed the functional observation battery data from the Sheets *et al.* (1997) study (previously discussed in 4. above) for evidence of CNS- or PNS-specific behavioural effects, but failed to find evidence of compounds that act solely on the PNS or CNS. This was partly due to the difficulty of separating effects (e.g. muscle weakness) that could equally be attributable to toxicity in either compartment. Therefore the hypothesis was rejected.

63. Rejection of these 6 alternate hypotheses led the Working Group to accept the initial hypothesis, that is, cholinesterase inhibition is the common mechanism of toxicity of the OP pesticides.

US EPA ORGANOPHOSPHATE COMMON MECHANISM GROUP

64. After evaluation of data on cholinesterase inhibition or symptoms of cholinergic toxicity on 40 US registered OP insecticides, these substances were placed in the US anticholinesterase CMG (Table 5). Since then the US EPA have used the CMG to establish a 'cumulative assessment group' for the OP insecticides. Some of the OPs (highlighted in bold in Table 5) in the CMG have been excluded from the cumulative risk assessment group because these OPs are either due to be phased out or exposures to these substances have been deemed to be negligible. Three compounds – chlorethoxyfos, phostebupirim and profenofos will only be examined qualitatively. The rest of the group form the Cumulative Assessment Group and will be used for cumulative risk assessment.

Table 5. List of substances placed in US OP common mechanism group

ORGANOPHOSPHATES	
Active	Reason for exclusion from cumulative risk assessment
Acephate	
Azinphos-methyl	
Bensulide	
Cadusafos	Zero-negligible exposures in individual risk assessments
Chlorpyrifos	
Chlorpyrifos-methyl	
Chlorethoxyfos*	
Coumaphos	Zero-negligible exposures in individual risk assessments
Diazinon	
Dichlorvos	
Dicrotophos	
Dimethoate	
Disulfoton	
Ethion	Due to be phased out
Ethoprop	
Parathion	Due to be phased out
Fenamiphos	
Fenitrothion	Zero-negligible exposures in individual risk assessments
Fenthion	
Fosthiazate	
Malathion	
Methidathion	
Methamidophos	
Parathion –methyl	
Mevinphos	
Naled	
Oxydemeton-methyl (ODM)	
Phorate	
Phosalone	
Phosmet	
Phostebupirim*	
Pirimiphos-methyl	
Profenofos*	
Propetamphos	Zero-negligible exposures in individual risk assessments
Sulfotep	Due to be phased out
Temephos	Zero-negligible exposures in individual risk assessments
Tetrachlorvinphos	
Tribufos	
Trichlorfon	

* these compounds will be examined qualitatively

ESTABLISHMENT OF ANTICHOLINESTERASE CMG FOR UK AUTHORISED OP PESTICIDES

65. Apart from phoxim, all of the OP pesticides currently authorised for use in the UK have been assessed by the US EPA (with US registration documentation and published studies) and included in the US anticholinesterase OP CMG. If the Science Group agrees with the direction taken by the US EPA and ILSI in establishing the OP CMG then it is proposed that the CMG be adopted for use in UK cumulative risk assessments.
66. Phoxim is a cholinesterase inhibitor known to cause cholinergic signs and symptoms. The European Union (EU) acceptable daily intake (ADI) of 0.00375mg/kg bw/day for phoxim is based on a NOAEL of 0.05mg/kg/day for brain cholinesterase inhibition and hepatotoxicity (CVMP, 1999). The basis of the OP CMG is: 1) the ability of the active to cause acetylcholinesterase inhibition and/or any of the signs or symptoms of cholinergic toxicity; 2) that this is the main toxic effect of the pesticides. Therefore, it is proposed that phoxim be grouped with the other OPs into a UK anticholinesterase OP CMG prior to any consideration of the inclusion of the N-methyl carbamates.
67. Substances comprising the proposed UK anticholinesterase OP CMG are shown in Table 6.

Table 6. List of substances in the Proposed UK OP CMG

ORGANOPHOSPHATES	
Active	Use
Azamethiphos	P, V, B
Chlorpyrifos	P, B
Chlorpyrifos-Methyl	P
Coumaphos	V
Diazinon	V
Fenitrothion	B
Fosthiazat	P
Malathion	P
Naled	V
Pirimiphos-Methyl	P, B
Phoxim	P, V
Tetrachlorvinphos	V

Key

P = used in agricultural pesticide products
V = used in veterinary medicine products
B = used in biocide products

ANNEX 2: N-METHYL CARBAMATES COMMON MECHANISM GROUP

(Yellow Paper)

REVIEW OF THE STRUCTURAL FEATURES OF THE N-METHYL CARBAMATES REQUIRED FOR ANTICHOLINESTERASE ACTIVITY.

Structural Consideration of the Carbamate Pesticides

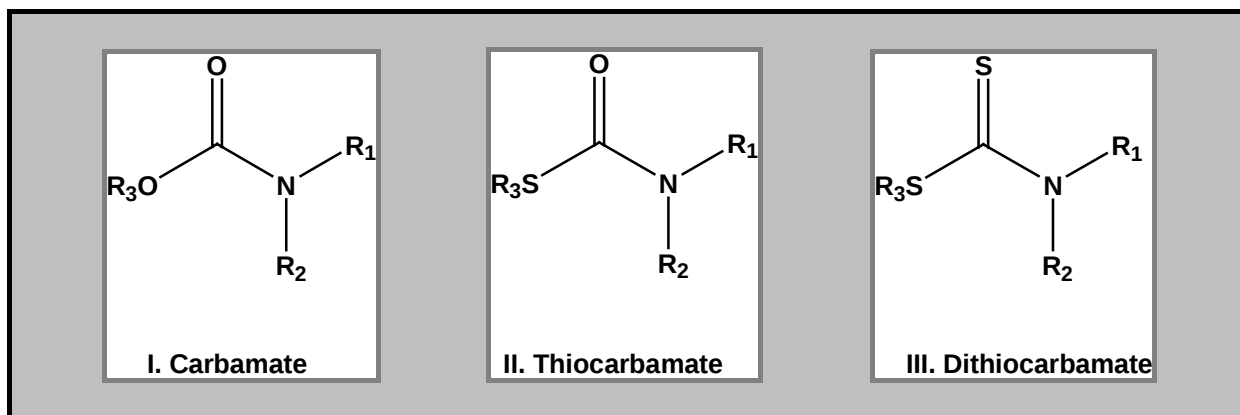


Figure 6. Schematic diagram of the structural backbone structures of the Carbamates, Thiocarbamates and Dithiocarbamates. (Reference: US EPA, Health Effects Division, Office of Pesticide Programs. 1999)

68. The carbamate pesticides were sub-divided into three main categories on the basis of:

- Whether the chemical linkages attached to the R₃ position was an oxygen or sulphur moiety;
- and:
- Whether an oxygen or sulphur was double bonded to the carbamate cation.

69. For any of these categories, R₃ esters may be an alkyl group, aryl group, oxime derivative or some other more complex chemical group.

70. This resulted in the initial establishment of three groups.

71. **Carbamates (N-methyl carbamates)**, which have an oxygen moiety attached at the R₃ position and whose carbamate cation is double bonded to oxygen. For carbamates, R₁ and R₂ may both be methyl groups (methyl carbamates – e.g. pirimicarb), or R₁ may be hydrogen and R₂ a methyl group (N-methyl carbamates – e.g. aldicarb). Carbamates which possess these small groups at the R₁ and R₂ position tend to be potent AChE inhibitors (e.g. aldicarb – Figure 7, and pirimicarb – Figure 8) and are marketed as insecticides. Carbamates whose R₁ and R₂ groups are generally larger than hydrogen or a methyl group tend to possess a much reduced, if at all present, anticholinesterase activity and are marketed as herbicides or fungicides.

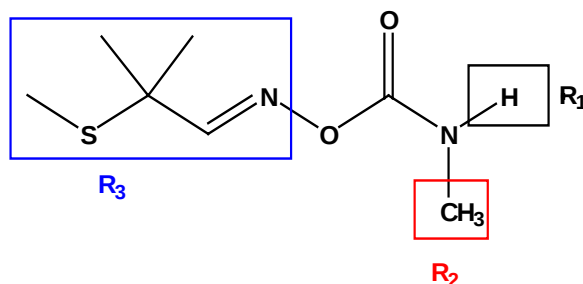


Figure 7. Structural diagram of aldicarb
(N-methyl carbamate)

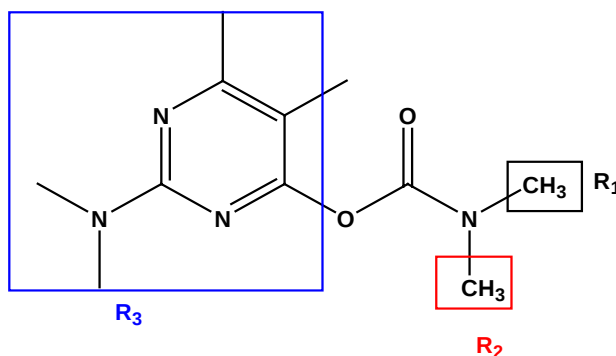


Figure 8. Structural diagram of Pirimicarb
(methyl carbamate)

72. **Thiocarbamates**, which have a sulphur atom attached at the R_3 position and whose carbamate cation is double bonded to oxygen. Thiocarbamates (e.g. thiobendacarb – Figure 9) are used as herbicides and possess the structural properties required to cause acetylcholine inhibition (i.e. possession of an R_3 ester that is capable of carbamylating the AChE hydroxyl serine residue in the active site). Thiocarbamates are far less potent AChE inhibitors than the group I carbamates although some may be capable of causing AChE inhibition in animals at high doses. However it is likely that for many thiocarbamates AChE inhibition is not their most sensitive effect.

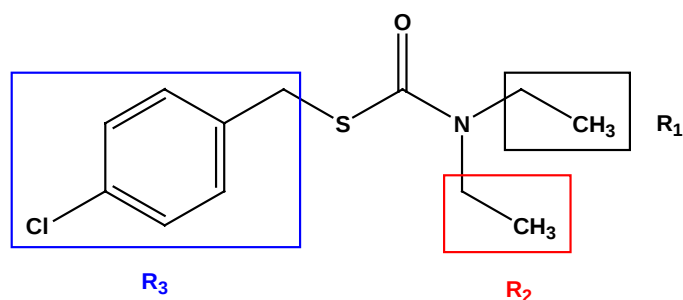


Figure 9. Structural diagram of thiobendacarb
(Thiocarbamate)

73. **Dithiocarbamates**, which have a sulphur atom attached at the R_3 position and whose carbamate cation is double bonded to sulphur. Dithiocarbamates (e.g. thiram – Figure 10) are also used as fungicides, and again few are also capable of causing AChE inhibition by virtue of the presence of an R_3 ester however AChE inhibition is generally at doses higher than other critical toxic effects.

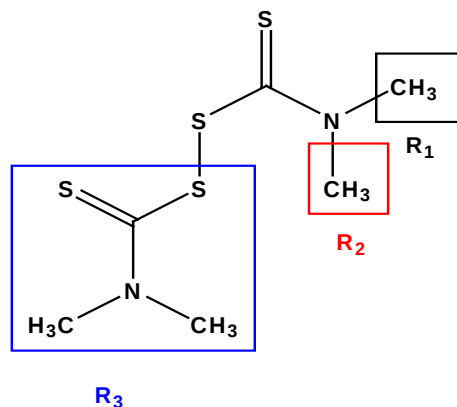
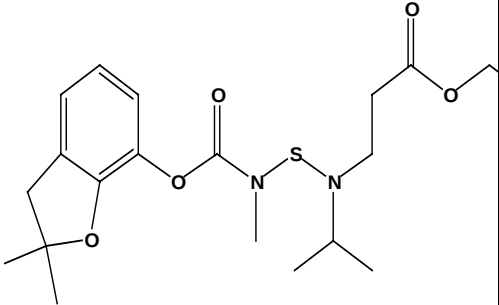
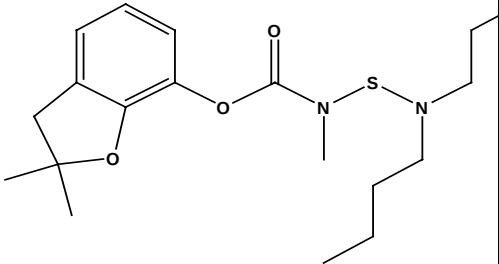
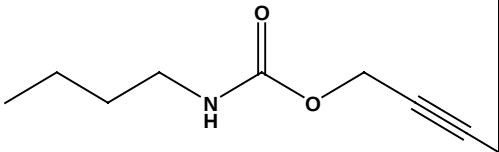
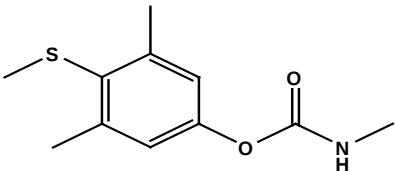
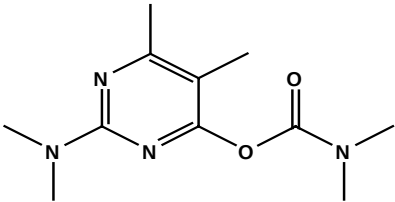
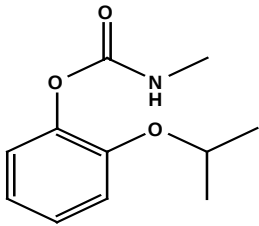


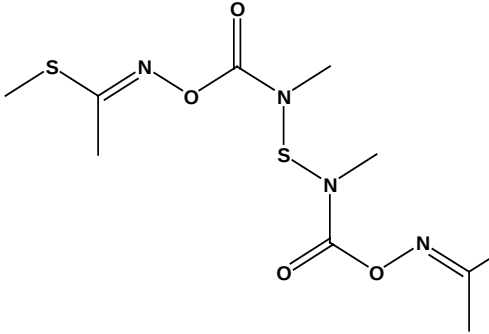
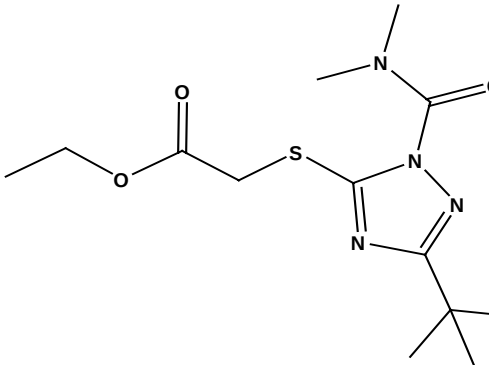
Figure 10. Structural diagram of thiram (Dithiocarbamate)

74. Table 6 shows the chemical structures of UK registered N-methyl carbamate insecticides.

Table 6. Chemical Structures of UK registered N-Methyl Carbamate Insecticides

Active	Uses	Molecular Structure
Aldicarb (P)	Insecticide	
Bendiocarb (P, B)	Insecticide	

Benfuracarb (P)	Insecticide Nematicide	
Carbosulfan (P)	Insecticide	
3-iodo-2-propynyl- n-butylcarbamate (B)	Wood Preservative, Surface biocide, Biocidal paint	
Methiocarb (P)	Nematicide	
Pirimicarb (P)	Insecticide	
Propoxur (V = companion species only)	Insecticide	

Thiodicarb (P)	Insecticide	
Triazamate (P)	Insecticide	

CRITICAL TOXIC EFFECTS OF THE CARBAMATE INSECTICIDES

75. The US EPA reviewed the main toxic effects of the three categories of carbamates and established that the main mode of toxic action (and pesticidal action) of the N-methyl carbamates is cholinesterase inhibition. The main mode of toxic action for most of the thiocarbamates and dithiocarbamates were independent of cholinesterase inhibition. These include reproductive toxicity, developmental toxicity, neuropathic effects and thyroid toxicity:
76. Reproductive Toxicity: decreased spermatogenesis, testicular toxicity, increased resorptions and post-implantation losses, and increases in spontaneous abortions.
77. Developmental Toxicity: cerebral and ocular malformations, morphometric alterations of the brain and skeletal anomalies.
78. Neuropathic Effects: sciatic nerve degeneration and neuronal cell necrosis.
79. Thyroid toxicity: disruptions in thyroid function associated with decreases in T3 and T4, increases in TSH, hypertrophy of thyroid tissue, thyroid hyperplasia and thyroid tumours.

MECHANISM OF TOXICITY OF THE N-METHYL CARBAMATES

80. The mechanism by which the N-methyl carbamates inhibit AChE is virtually identical to the mechanism of OP induced cholinesterase inhibition (Figure 11). There are, however, two distinct differences in the reaction between the enzyme and these two classes of compounds:

- Optimal reactivity of the OP is essential for high anticholinesterase activity for the OPs, whilst a 'good fit' of the carbamate to the active site of the enzyme is essential for high AChE activity for the carbamates.
- The spontaneous regeneration of the carbamylated enzyme to its fully active state is rapid compared to the spontaneous regeneration rate of the phosphorylated enzyme. This factor has a significant impact on the recovery rate and the clinical features of recovery. The half-life for recovery of the carbamylated enzyme is approximately 30 minutes with full recovery from adverse clinical signs and symptoms over 8 hours. During the 8-hour period signs of progressive recovery are usually evident. Phosphorylated enzymes, can depending on the specific active, remain inhibited over a period ranging from days to weeks. Clinical signs of recovery occur after the inhibition is terminated.

Biochemistry of N-Methyl Carbamate Anticholinesterase Activity

81. The binding affinity of the N-methyl carbamates to the enzyme determines the compound's ability and potency as a cholinesterase inhibitor. As with the Ops, anticholinesterase activity of N-methyl carbamates is a two-step process, the first being the formation of an enzyme inhibitor complex and the second being the carbamylation of the enzyme (Figure 12).

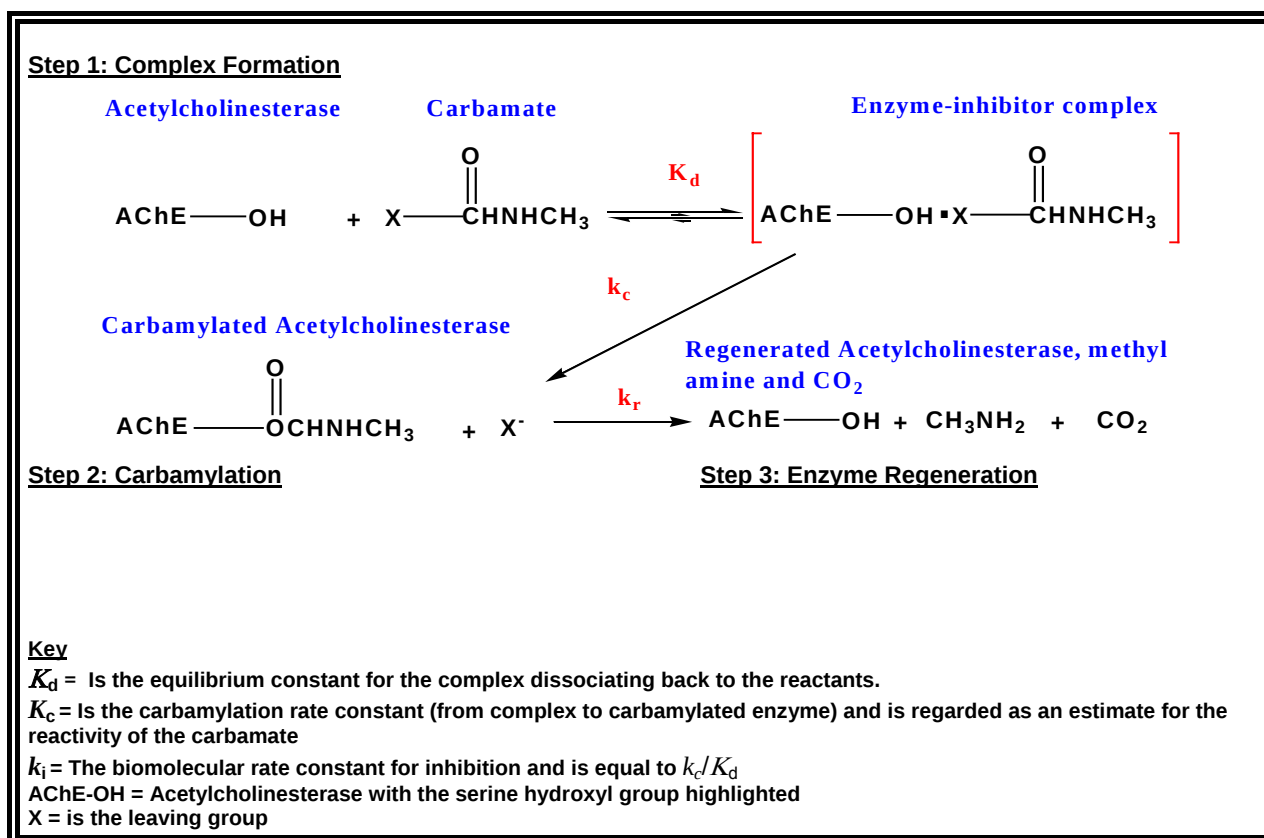


Figure 11. Chemical equation of N-methylcarbamate inhibition of AChE (Fukuto, 1990)

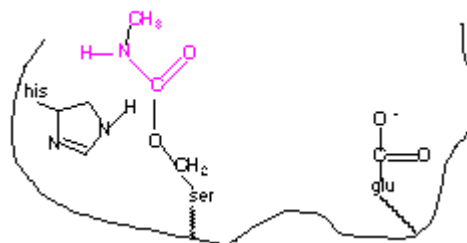


Figure 12. Diagram of an AChE phosphorylated by a carbamylated insecticide

82. Whilst the chemical reactivity of the OP is the most important factor in its ability to inhibit AChE and is the driving force that determines its potency, the opposite is the case for the carbamates. The single most important element for methyl carbamate esters to cause cholinesterase inhibition is their binding affinity to the active site of the enzyme i.e. form a stable enzyme inhibitor complex prior to carbamylation. This factor determines their ability to inhibit the cholinesterases and their potency as inhibitors. The carbamates are intrinsically reactive so they, unlike the OPs, do not require an electron withdrawing substituent in the aryl moiety for high anticholinesterase activity. Indeed the introduction of a strong electron withdrawing substituent would make the carbamates so reactive that they would be hydrolytically degraded before enzyme inhibition could occur. The kinetics of carbamylation and enzyme regeneration are significantly more rapid than the kinetics of phosphorylation.

THE OFFICE OF PESTICIDE PROGRAMS POLICY (OPP) ON THE GROUPING OF CARBAMATE PESTICIDES AND GROUPING OF AChE INHIBITING CARBAMATES AND ORGANOPHOSPHATES

83. Upon review of the structural requirements, mechanism of toxicity, toxic effects and pesticidal action of the carbamate compounds, the US EPA Office of Pesticides Programs (OPP) addressed the following questions as a means of deciding whether to group the carbamate pesticides and group the cholinesterase inhibiting carbamates with the OPs:

- Does acetylcholinesterase inhibition provide sufficient evidence of a common mechanism of toxicity for grouping of carbamate pesticides?
- Can carbamate pesticides be sub-grouped based on the characteristic of some to produce effects unrelated to cholinesterase inhibition?
- Should the carbamate pesticides that inhibit AChE be grouped with the OP pesticides that inhibit AChE?

84. Does acetylcholinesterase inhibition provide sufficient evidence of a common mechanism of toxicity for grouping of carbamate pesticides?

The common toxic effects of the cholinesterase inhibiting carbamates are AChE and/or cholinergic effects. OPP concluded that AChE inhibition was sufficient evidence of a common mechanism of toxicity for the grouping of AChE inhibiting carbamate pesticides. The common mechanism of toxicity of these compounds is the carbamylation of the hydroxyl serine residue in the active site of AChE which leads to an accumulation of ACh at a neuromuscular junction/nerve synapse or other neuroeffector junction (e.g. gland) which, if sustained or at a high dose, will eventually lead to adverse effects on the cholinergic system. This would manifest in the following clinical signs: nausea, vomiting, gastrointestinal distress, tremors, paralysis and depression of respiratory function.

85. Can carbamate pesticides be sub-grouped based on the characteristic of some to produce effects unrelated to cholinesterase inhibition?

The OPP concluded that the toxicity profiles of each carbamate would need to be examined thoroughly in order to identify chemicals that belong to CMG. Carbamates should not be grouped with other cholinesterase carbamates unless there is sufficient and reliable data to show that they can inhibit AChE. They also concluded that carbamates, whose most sensitive toxicological effects are anything other than cholinesterase inhibition, should be grouped with other carbamates if there is sufficient evidence that they share a common mechanism of toxicity for these other(s) effects.

86. Should the carbamate pesticides that inhibit AChE be grouped with the OP pesticides that inhibit AChE?

The OPP concluded that cholinesterase inhibiting carbamates and OPs do share a common mechanism of toxicity and should be grouped during the initial phase of determining the cumulative risk assessment for the group. The two classes of substances act as pseudo substrates to ACh, cholinesterase inhibition is their main mode of pesticidal action, and they both produce the same clinical and neurobehavioral effects in animals and humans.

87. The OPP decided to establish the N-methyl carbamates as a CMG on the basis of cholinesterase inhibition and agreed to work towards establishing separate CMGs for the thiocarbamates and dithiocarbamates. They did not, however, group the N-methyl carbamates with the OPs despite recommendations from the Scientific Advisory Panel (the main peer review committee for the EPA) for reasons that will be discussed in Annex 3. The CMG for the N-methyl carbamates is listed in Table 7.

Table 7. US EPA N-Methyl Carbamate Common Mechanism Group

N-METHYL CARBAMATES
Aldicarb Asulam Bendiocarb Carbaryl Carbofuran Chlorpropham Desmidipham Formetanate Methiocarb Methomyl Oxamyl Phenemedipham Pirimicarb Propamocarb Propham Propoxur Thiodicarb Thiophanate

**ANNEX 3. REVIEW OF THE US EPA's DECISION TO GROUP THE
ANTICHOLINESTERASE OPs AND N-METHYL CARBAMATES SEPARATELY**

(Green paper)

CONCLUSIONS ARISING FROM AN INTERNAL CONSIDERATION OF THE N-METHYL CARBAMATES AND OP SUBSTANCES

88. The decision to group the two classes of substances together was considered during an internal US EPA review of the carbamates and the following was concluded:
89. 'In conclusion, while the biochemical reaction of the different chemical structures of these classes is different, i.e., carbamylation versus phosphorylation, and the physiological consequence, inhibition of AChE activity, is essentially the same. Because the duration of inhibition and effects may overlap between these two classes, there is no clear means of separating these two groups with respect to potential interactions. In the context of a common mechanism of toxicity and the criteria defined in US EPA's *Guidance For Identifying Pesticide Chemical and Other Substances that have a Common Mechanism of Toxicity* (US EPA, 1999a), OPP knows of no means to meaningfully differentiate between the consequences of AChE inhibition by these carbamates and OP compounds'.
90. However the US EPA has since established separate CMGs for the OPs and N-methyl carbamates.
91. The US EPA's decision to group the N-methyl carbamates and OPs into separate CMGs was based on the differences between the kinetics of phosphorylation of acetylcholinesterase and carbamylation. In a telephone conversation with an employee of the US EPA (Mr Karl Baetaki) it was stated that the US EPA was currently prioritising individual groups and working towards establishing cumulative risk assessments for them before looking at 'cross' grouping substances. The decision not to group the N-methyl carbamates with the OPs was to do with the pharmacokinetic differences between carbamylation and phosphorylation (i.e. recovery from carbamylation is within 8 hours which is significantly shorter compared to the recovery rates from phosphorylation). The US EPA are considering whether to look at the two groups in the future but as yet no decisions have been made.
92. There is extensive data from animal studies as well as some human studies, which shows that the N-methyl carbamates and the OPs share the ability to **a)** inhibit acetylcholinesterase (which importantly is their main mode of toxicity), and **b)** can cause toxic effects on cholinergic function. The only difference between the actions of the two classes of pesticides are: 1) the biochemical reaction leading to enzyme inhibition (phosphorylation of the hydroxyl serine residue in the active site of the AChE enzyme in the case of the OPs as opposed to carbamylation of the serine residue in the case of the carbamates); 2) the rate of regeneration of the carbamylated enzyme (which is much faster than the regeneration of the phosphorylated enzyme).
93. It is acknowledged that the degree of toxicity and the spectrum of cholinergic effects elicited by individual members of each class can vary due to differences in toxicokinetic and toxicodynamic parameters and potencies. Nevertheless if an individual is exposed to an N-methyl carbamate whilst still suffering the effects from a prior OP exposure (or vice versa), there is a chance that the presence of the two anticholinesterase compounds will lead to an increase in the amounts of AChE

being inhibited. In turn, this will lead either to more severe toxic signs in the compartments already affected and/or an increase in the number of compartments affected. This is likely to be a dose additive effect. The fact that there is potential for concurrent exposures to both classes of pesticides to occur from various sources in the diet, for example, makes the decision by the US EPA to group the OP and N-methyl carbamates into separate CMGs highly questionable particularly as both groups have been established on the basis of the same common mechanism of action (e.g. AChE inhibition) and the same common toxic effect (e.g. adverse cholinergic function).

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